A rapid throughput technique to isolate pyrogenic carbon by hydrogen pyrolysis for stable isotope and radiocarbon analysis

Jordahna Haig[1], Philippa L. Ascough[2], Christopher M. Wurster[1], Michael I. Bird[1]

[1]College of Science and Engineering, ARC Centre of Excellence of Australian Biodiversity and Heritage and Centre for Tropical Environmental and Sustainability Science, James Cook University, Cairns, QLD 4870, Australia

[2]NERC Radiocarbon Facility, SUERC, Scottish Enterprise Technology Park, Rankine Avenue, East Kilbride, G75 0QF, UK

Keywords: Radiocarbon pretreatment; pyrogenic carbon; hydrogen pyrolysis; charcoal

Abstract

RATIONALE: Rapid, reliable isolation of Pyrogenic Carbon (PyC; char, soot, black carbon; biochar) for determination of stable carbon isotope ($\delta^{13}C$) composition and radiocarbon (^{14}C) dating is needed across multiple fields of research in geoscience, environmental science and archaeology. Many current techniques do not provide reliable isolation from contaminating organics and/or are relatively time consuming to employ. Hydrogen pyrolysis (HyPy) does provide reliable isolation of PyC but the current methodology is time consuming.

METHODS: We explored the potential for subjecting multiple samples to HyPy analysis by placing up to nine individual samples in custom designed borosilicate sample vessels in a single reactor run. We tested for cross contamination between samples in the same run using materials with highly divergent radiocarbon activities (~0.04 to 116.3 pMC), $\delta^{13}C$ values (-

11.9 to -26.5‰) and labile carbon content. We determined $^{14}C/^{13}C$ by accelerator mass spectrometry and $\delta^{13}C$ values by elemental analyser coupled to continuous flow isotope ratio mass spectrometer.

RESULTS: Very small but measurable transfer between samples of highly divergent isotope composition was detectable. Where samples are of broadly similar composition, this cross contamination is considered negligible with respect to measurement uncertainty. Where samples are of divergent composition, it was found that placing a sample vessel loaded with silica mesh adsorbent between samples eliminated measurable cross-contamination in all cases for both $^{14}C/^{13}C$ and $\delta^{13}C$ values.

CONCLUSION: It is possible to subject up to seven samples to HyPy in the same reactor run for determination of radiocarbon content and $\delta^{13}C$ value without diminishing the precision or accuracy of the results. This approach enables an increase in sample throughput of 300-600%. HyPy process background values are consistently lower than the nominal laboratory process background for quartz tube combustion in the NERC Radiocarbon Laboratory, indicating that HyPy may also be advantageous as a relatively 'clean' radiocarbon pretreatment method.

1. Introduction

Pyrogenic carbon (PyC, also known as char, black carbon, biochar) is derived from the incomplete combustion (pyrolysis) of organic matter during natural fires, the purposeful pyrolysis of biomass to create 'biochar', or via fossil fuel combustion [1,2]. PyC is a ubiquitous component of carbon in soils, sediments, atmospheric particulates, fresh and marine waters, in both dissolved and/or particulate (microscopic and macroscopic) forms.

PyC is important as a poorly understood, slow-cycling component of the global carbon cycle, that is now known to comprise a significant, but as yet unquantified, pool that contains some of the most recalcitrant organic carbon on Earth [3,4]. In the form of biochar, PyC also has the potential to sequester significant amounts of carbon over time periods far exceeding that of biomass, therefore offsetting at least a proportion of current anthropogenic CO_2 emissions [5]. PyC is also useful as a form of recalcitrant carbon that can provide a valuable palaeoenvironmental proxy through its stable isotope composition [6]. It is one of the most common materials (as 'charcoal') used for construction of radiocarbon chronologies in support of archaeological and Quaternary environmental studies.

Quantification and isolation of PyC has long proven problematic. This is because PyC represents a continuum of complex molecular components with differing chemical and spectroscopic properties. Each method targets a particular 'window' along this continuum, meaning the 'PyC' isolated by two differing methods can be chemically quite distinct. Thus, while many techniques have been developed to analyse PyC in a range of environmental matrices, these techniques produce widely divergent results for the same samples, the same technique has produced divergent results across different laboratories and some techniques are not broadly applicable across the range of matrices that can contain PyC [7]. The key difficulty however, is that many methods rely on an operational definition of PyC in order to achieve isolation. This often requires that what remains after the process must, by definition, be PyC, and that quantification relies upon a single parameter, such as weight loss during processing. Problems arise if non-PyC material is not removed e.g. due to hydrophobic protection of plant waxes in aqueous solutions [8], or if PyC is formed *de novo* during oxidative reactions [9]. Procedures isolating PyC on a secure chemically-defined basis, are therefore preferable.

Hydrogen pyrolysis (HyPy) was originally used as a method for the efficient conversion of macromolecular organic matter to dichloromethane soluble oils with conversions near 100% possible using high hydrogen pressures (>10 MPa) at high temperature [10]. During these experiments, it was found that in some cases substantial amounts of highly aromatic macromolecular carbon remained in the residues, attributed to PyC [11].

The possibility that HyPy could be used to quantify PyC was first discussed by Ascough et al [12,13], who developed a hydrogen pyrolysis procedure specific to eliminating labile components and leaving only polyaromatic carbon in the residue. Meredith et al [14] demonstrated that HyPy yielded results within the range of other techniques for PyC in a range of matrices using the black carbon ring trial samples of Hammes et al [7]. Meredith et al [14] also demonstrated that HyPy was able to remove potentially interfering non-pyrogenic materials with the exception of anthracite, and that the component isolated by HyPy is chemically highly consistent, being polyaromatic carbon with a ring size greater than 7 (coronene). Smaller pyrogenic polyaromatic molecules can be collected by cryogenic trapping downstream of the HyPy reactor for separate quantification if required [15-17]. More recent work has demonstrated the utility of HyPy analysis across a range of environmental matrices, for simple quantification [18-20] with good reproducibility within and between laboratories [21], as well for stable isotope analysis [6,22,23] and radiocarbon dating [12,13,24,25].

While the advantages of HyPy for the isolation and quantification of a well-defined component of PyC have now been clearly demonstrated, the technique remains relatively slow. The instrument reactor conventionally accommodates one sample at a time and a single run takes approximately 43 minutes. While the technique is not more time consuming than many other competing techniques, the current throughput limits the scale of projects that can be undertaken. At the same time, research interest in PyC is growing rapidly, particularly for

carbon-cycle science applications. For example, measuring PyC abundance and turnover times by ^{14}C, which by their nature require high-volume throughput. The general need to improve throughput is now driving modifications to other established techniques such as BPCA analysis [26].

Here we report on the results of experiments designed to test whether multiple samples can be run simultaneously in a single HyPy reactor for accelerated throughput and efficiency, without either cross contaminating the samples or degrading the accuracy or precision of individual stable isotope and radiocarbon analyses.

2. Materials and Methods

2.1 Samples

The samples for this study were chosen to provide the largest possible contrast in lability of material (i.e. degree of aromaticity), and in carbon isotope ($\delta^{13}C$ and ^{14}C) composition. Five matrixes were chosen for experiments designed to test the degree to which combining multiple samples with divergent characteristics in a single reactor run effected the measured PyC abundance, $\delta^{13}C$ and ^{14}C measurements of individual samples in the same reactor (see Table 1.). Throughout this manuscript, error is reported as 2σ.

Three materials of known radiocarbon content were chosen for experiments designed to test, 1) the ^{14}C background of the HyPy process itself, and 2) the degree of inter-sample transfer of carbon when combining multiple samples with different ^{14}C content in a single HyPy run:

(i) *TIRI barley mash (TBM):* This standard originates from the Third International Radiocarbon Intercomparison [27]. It is composed of lignocellulosic biomass, known to be entirely labile during HyPy. The consensus value for TBM is 116.35 ±0.016 pMC. TBM was used as a source of excess labile C from a sample in HyPy, which thus had the potential to be

transferred to other samples in the same HyPy reactor. The relatively high ^{14}C content of TBM makes it possible to detect even small quantities of cross-contamination, when used with a contrasting RCD (radiocarbon dead) material (below).

(ii) *Anthracite*: This is a NERC Radiocarbon Facility in-house process standard anthracite [28]. It is a highly aromatic material, and anthracite coal is known to be resistant to HyPy [14]. This material is 'radiocarbon-dead', being much greater than 50,000 years in age, and is in use as a process background material for radiocarbon analyses. For the quartz tube combustion method of CO_2 production (the process applied in this study), the long-term average background value at the NERC Radiocarbon laboratory is 0.17 ±0.02 pMC. The anthracite standard was used to identify whether the HyPy ^{14}C background was commensurate with this value before, during, and after the experiments described below, and to provide confidence that any observed variation in ^{14}C during multiple simultaneous HyPy runs can be ascribed to a specific process (i.e. transfer of carbon between sample vessels within the reactor for this specific experiment), and not an inherent feature of the method itself.

(iii) *RDC*: This is a radiocarbon-dead charcoal produced experimentally from a log of Miocene age and previously characterized by Bird et al [29]. It is a material resistant to conversion during HyPy, with a radiocarbon activity equivalent to 0.04 ±0.02 pMC. This material is also highly aromatic, similar to the anthracite coal standard, but was included as it represents a more typical form of natural PyC (i.e. the product of biomass that has been thermally altered during fire). During the experiments below, RDC was used to represent material into which the introduction of cross-contamination (from TBM) during HyPy could be monitored.

2.2 Hydrogen pyrolysis

Hydrogen pyrolysis has been described in detail in a number of publications [10,30,12,14]. Briefly, 25-100 mg aliquots of each sample were loaded with a Mo catalyst using an aqueous/methanol (1:1) solution of ammonium dioxydithiomolybdate [$(NH_4)_2MoO_2S_2$]. Catalyst weight was ~10% sample weight for all samples to give a nominal loading of ~1% Mo. Catalyst loaded samples were then lyophilized and weighed aliquots of each sample were loaded into small bespoke borosilicate sample vessels of 7 mm outer diameter, 1 mm wall thickness and 10 mm (small) or 15 mm (large) overall length. The base of each sample vessel was fitted with a porosity #1 silica frit to allow gas throughflow (manufactured by Robson Scientific, Sawbridgeworth, UK). Small glass microfiber filters ~5mm in diameter were used to line the bottom of the vessel and plug the top of the vessel to keep the sample in place. These are hole-punched using a cork borer from standard Whatman 0.45 µm glass microfiber filters.

In order to facilitate higher throughput (i.e. >1 sample per reaction) the HyPy reactor setup was modified from that outlined in Ascough et al [12]. In this study, the ¼″ silica trap has been removed and the standard 254mm 9/16″ reactor has been replaced with a 475mm nipple (see Fig. 1). The upper 196mm of the nipple now functions as the reactor while the bottom 279mm is filled with silica mesh and used as a trap to collect the products of the reaction (i.e. the labile carbon fraction). This larger trap does not require cleaning for upwards of 45 reactions.

Multiple samples (4-9) were loaded one above the other into the HyPy reactor as per the experimental design outlined in Figure 2. After sample loading, the reactor was pressurized with hydrogen to 15 MPa with a flow rate of 5 L min^{-1}, then heated using a pre-programmed temperature profile. We used the recommended temperature program previously optimized for PyC quantification where samples are initially heated at a rate of 300°C min^{-1} to 250°C, then at a rate of 8°C min^{-1} until the final hold temperature of 550°C for 5min [12,14].

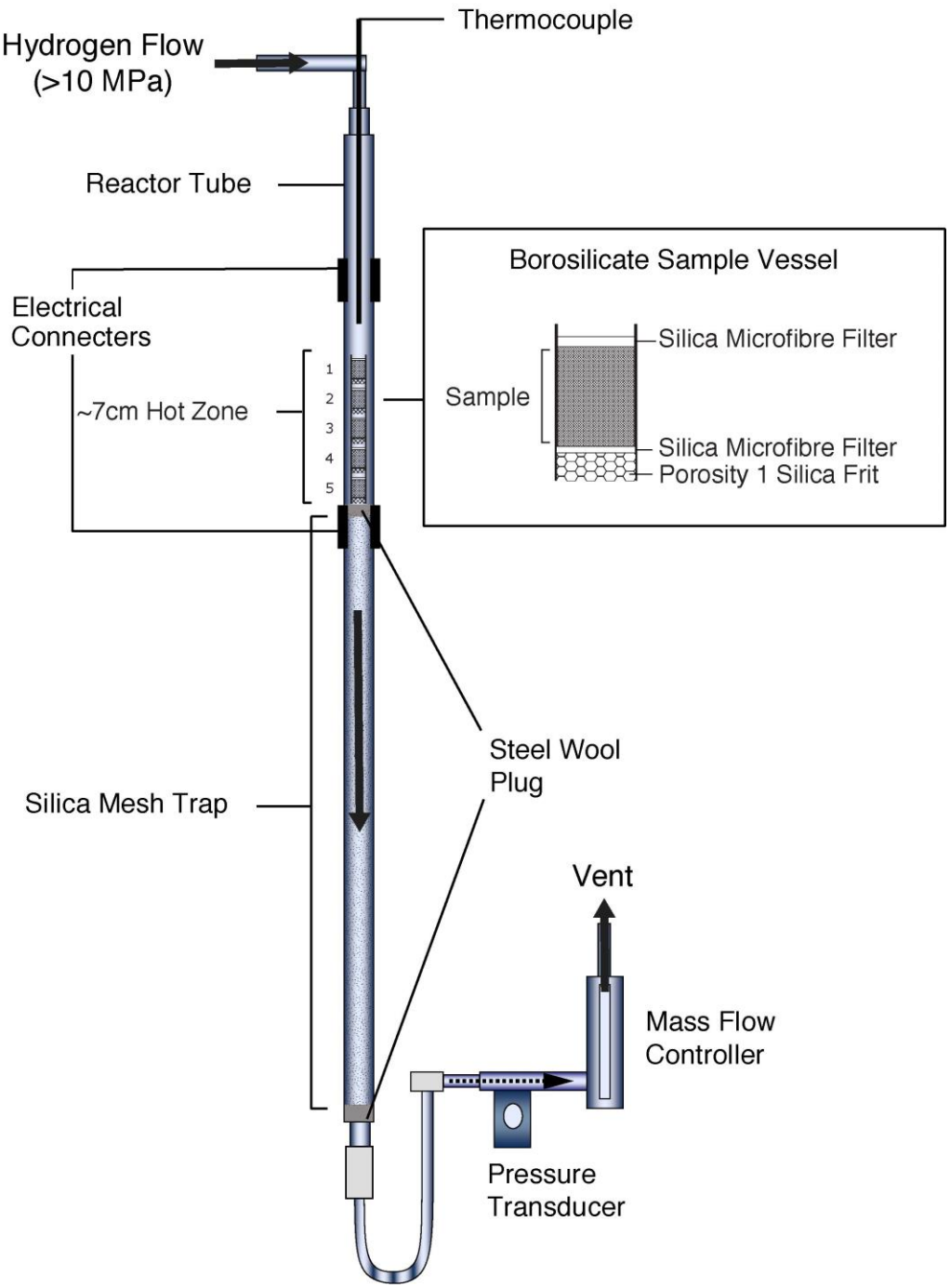


Figure 1. *Hydropyrolysis reactor schematic showing placement and design of the borosilicate sample vessels, steel wool placeholder, silica trap and direction of Hydrogen flow.*

2.3 Carbon abundance and stable isotope composition

Carbon abundances and isotope compositions of all samples were determined using a Costech Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) fitted with a zero-blank autosampler coupled via a ConFloIV (Thermo Fisher Scientific, Waltham, MA, USA) to a ThermoFinnigan DeltaV^{PLUS} using Continuous-Flow Isotope Ratio Mass Spectrometry (EA-IRMS) at the Advanced Analytical Unit at James Cook University, Cairns. Stable isotope results are reported as per mil (‰) deviations from the VPDB reference standard scale for $\delta^{13}C$ values. Precisions (2σ) on internal standards were better than ±0.2 ‰. Because the catalyst undergoes (~25%) weight loss during HyPy, the abundance of residual carbon in the sample after hydrogen pyrolysis is determined as the mass of carbon after treatment relative to the mass of carbon loaded and the results reported as the residual carbon present in the sample (C_R– residual carbon not removable by HyPy). Reproducibility is considered to be 2% of the value, based on repeated analyses [31].

2.4 Radiocarbon measurement

HyPy residues were converted to CO_2 by combustion in sealed quartz tubes, and the evolved gas was cryogenically purified and converted to graphite for analysis using Fe/Zn reduction [32]. Sample $^{14}C/^{13}C$ ratios were measured by Accelerator Mass Spectrometry [29] at the Scottish Universities Environmental Research Centre. Measured $^{14}C/^{13}C$ ratios were normalized to a $\delta^{13}C$ value of -25‰ and expressed as % modern carbon (pMC) according to Stuiver and Polach [33].

2.5 Experimental Design

The experimental design is given in Figure 2, it comprises four sets of experiments using the materials described in table 1.

196 (i) *Experiment 1:* The purpose of this experiment was to determine the location of the
197 reactor 'hot zone' i.e. the region where the temperature is consistent and hydrogen pyrolysis
198 is effective and reproducible. This zone defines the maximum number of samples that can be
199 run simultaneously using the modified reactor setup. Initially, 9 small (10 mm long) sample
200 vessels containing ~100mg of a reference material (BCM) were run in quadruplicate. This
201 experiment was repeated with 6 large (15 mm long) sample vessels of a composite soil
202 control sample (AGR), also run in quadruplicate.

203 (ii) *Experiment 2:* In this experiment, (fig 2a), ~30mg of anthracite was first used to
204 determine the HyPy ^{14}C background for the radiocarbon experiments. Measurements of
205 anthracite were also performed before and after a HyPy run, to test for any sequential changes
206 in the HyPy instrument ^{14}C background. Two RDC samples were used as controls. In
207 experiment 2a, ~100mg (replicate 1) or ~50mg (replicate 2) of TBM was placed vertically
208 above three RDC samples in the same reactor. The TIRI standard and three RDC samples
209 were all contained in the same HyPy reactor run to test whether labile carbon is transferred
210 from the sample above to the samples below, in the direction of hydrogen flow, manifest in a
211 measurable increase in radiocarbon in the RDC samples. This experiment was repeated in
212 experiment 2b, with the addition of a sample vessel filled with 70-200μm silica mesh
213 between the ~50mg of TBM and RDC samples, replicated twice.

214 *Experiment 3:* The purpose of this experiment was to quantify the amount of labile TOC
215 is transferred 'downstream' from one vessel to another over the course of a single reactor run.
216 To do this, 2-10mg of carbon (in the form of Sugarcane Leaves or BCM) was placed in a
217 sample vessel above a series of four silica mesh spacers, with or without an empty sample
218 vessel (acting as a spacer) at position 2. The silica mesh was analysed for C abundance by
219 Costech elemental analyser immediately following the HyPy. In addition, a background silica

mesh carbon abundance was determined by successive HyPy runs comprising exclusively silica mesh spacers.

Experiment 4: The purpose of this experiment was to test the degree to which labile carbon released from one sample during a HyPy run, is transferred to another 'downstream' sample within the HyPy reactor. In this experiment an inhouse reference of labile C_3(C_4) material, C_3 rainforest leaves (or C_4 sugarcane leaves) was placed in a sample vessel above a vessel loaded with a C_4 (or C_3) soil sample containing PyC of known abundance and stable isotope composition, both with and without a silica mesh spacer at position 2 (fig 2d). This experiment was repeated in quadruplicate.

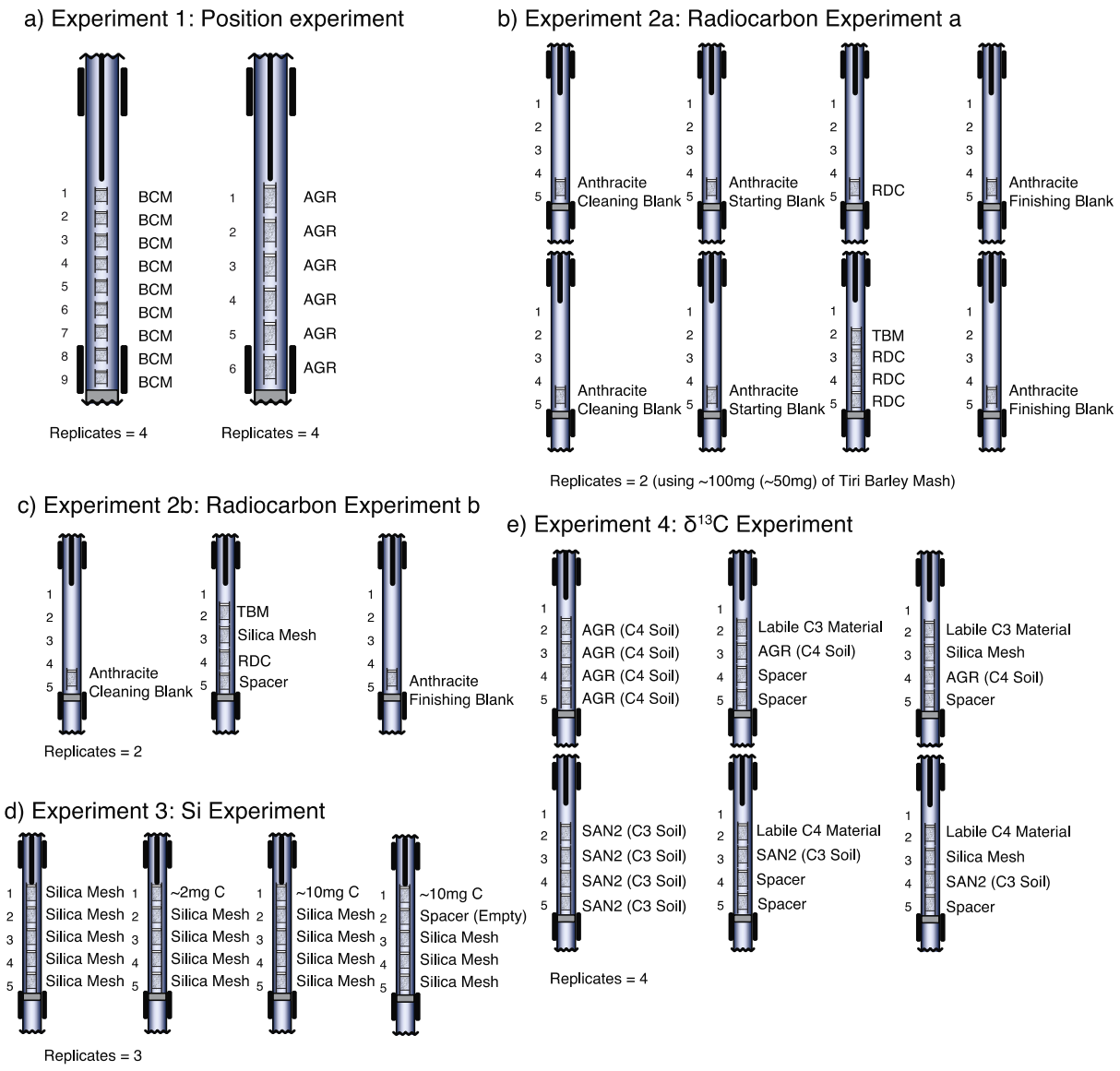


Figure 2. *Experimental design, showing the position and contents of the sample vessels in each HyPy experiment run, the number of replicates and the order of runs. A) position experiment to determine the ideal number of samples per run and the location of the 'hot zone' within the reactor; B) radiocarbon experiment A, to determine if running multiple samples simultaneously leads to ^{14}C contamination of samples below; C) radiocarbon experiment B, to determine if adding silica mesh spacers between samples reduces ^{14}C contamination of the sample below; D) silica mesh experiment to quantify the transfer of labile C down the profile, with or without an empty sample vessel (spacer) E) Replication of the previous experiments to test whether typical C_3 (C_4) soil samples are contaminated by labile C_4 (C_3) material from the vessel directly above, and if a silica mesh spacer resolves this.*

Pairwise multiple comparison tests were used in experiment 1 to determine whether PyC% was influenced by position within the HyPy reactor, and to identify the area within the reactor in which the variability was least (i.e. the 'hot zone') and thus the ideal positioning of the steel wool plug that holds all the vessels in place in the reactor (Figure 1). Pairwise multiple comparison tests were also used in experiment 4 to determine whether the amount of labile carbon above a sample impacts the $\delta^{13}C$ value of PyC in a sample downstream.

3. Results and Discussion

3.1 Experiment 1: 'hot zone' delimitation

In conventional HyPy, samples are processed individually in a 43 minute HyPy run [12,14]. To determine the feasibility of running multiple samples simultaneously, multiples of 9 small sample vessels (10 mm; n = 26 total) or 5 large sample vessels (15 mm; n = 20 total) stacked on top of each other, were run within the same reactor in quadruplicate runs to

254 determine the maximum number of samples that can be run in tandem without compromising
255 precision. Across all positions PyC ranged from 0.2 - 1.1% (0.1 - 0.2%) and δ^{13}PyC ranged
256 from -26.3 to -26.6‰ (-15.4 to -14.2‰) for the experiments based on BCM (AGR). The
257 summary statistics are given in table 2.

258 Comparison of the results by position in the reactor (fig 3a) shows that results from the 10mm
259 vessels (BCM) in position 8 and 9 do not belong to the same population as samples 1-7,
260 indicating that the 'hot zone' is within ~7cm of the thermocouple tip in the reactor (μ = 0.3
261 ±0.08%, -26.5 ±0.6‰), below this reproducibility is diminished. A repeat of the same
262 experiment using 15mm vessels (fig 3c: AGR) confirms the ~7cm hot zone as there is no
263 statistical difference in PyC% between samples in positions 1-5 i.e. within ~7.5cm of the
264 thermocouple (μ = 0.2 ±0.01%, -14.7 ±0.3‰). Position affects PyC% to a greater extent than
265 δ^{13}PyC (fig 3b and 3d) as there is no statistical difference between any position when using
266 either small or large vessels providing an error (2σ) of 0.6‰ and 0.3‰ is acceptable. This
267 confirms that multiples of 7 (small 10 mm vessels) or 5 (large 15 mm vessels) samples can be
268 accurately run together within a single 45 minute HyPy run, with a precision better than
269 0.08% PyC and 0.6‰ δ^{13}PyC, equating to a 400-600% increase in throughput compared to
270 the conventional 'single sample per run' method.

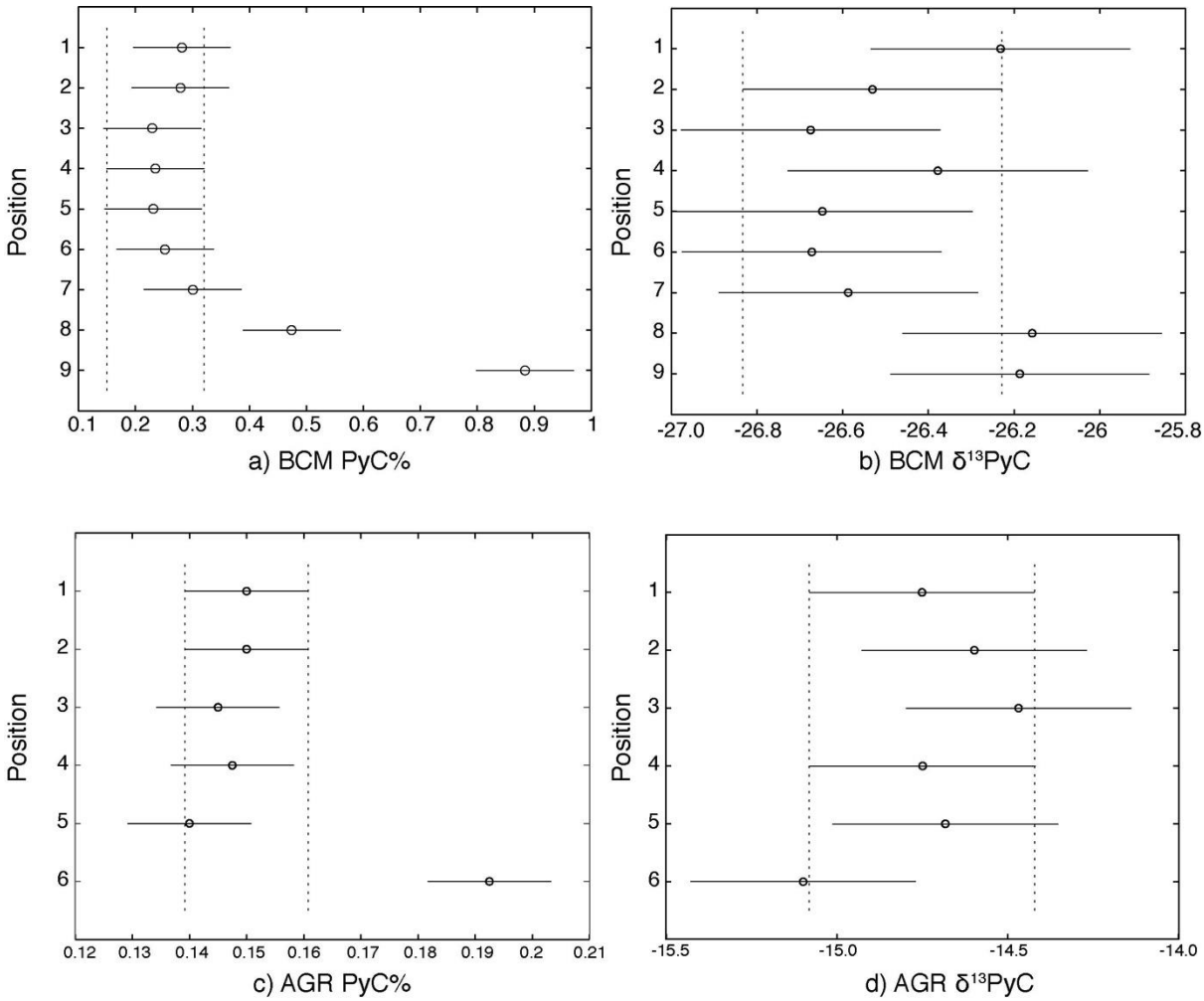


Figure 3. *Pairwise multicomparison plot showing test estimates i.e. group mean μ (circles), and comparison intervals α (lines) for 10mm sample vessels of BCM PyC% (A) and δ^{13}PyC (B); and 15mm sample vessels of AGR composite soil PyC% (C) and δ^{13}PyC (D).*

3.2 Experiment 2a: Radiocarbon pretreatment background

HyPy has already been successfully applied as a rapid pretreatment method for isolating and purifying PyC for ^{14}C measurements [12,13,24,25]. Experiment 1 confirmed that it is statistically acceptable to process up to 5 large samples in tandem for PyC quantification by HyPy. In experiment 2, we assess the potential for pre-treating multiple samples simultaneously when radiocarbon measurement of these samples is also required. To determine the appropriate HyPy radiocarbon pretreatment background, a series of anthracite cleaning, and finishing blanks were run in isolation before and after each experiment.

283 Combining all anthracite runs indicates a HyPy background value of 0.13 pMC (μ = 0.10, 2σ
284 = 0.03, n = 11). This is significantly less than the standard internal NERC Radiocarbon
285 Laboratory quartz tube process background value of 0.17±0.02 pMC. The anthracite finishing
286 blanks run immediately after each experiment were indistinguishable from the lab process
287 background (μ = 0.10, σ = 0.04), meaning there is no detectable additional ^{14}C added by the
288 HyPy process above that of the quartz tube combustion when compared to standard process
289 background values.

290 These results indicate that normal cleaning between HyPy runs (i.e. manual rinsing of the
291 reactor with dichloromethane, followed by drying of the reactor and complete removal of
292 dichloromethane during the warm-up phase of HyPy treatment, is sufficient to preserve
293 appropriate ^{14}C background values. Indeed, the measured HyPy process background values
294 were consistently lower than the nominal laboratory process background for the quartz tube
295 method of combustion, indicating that the HyPy process may be a particularly 'clean'
296 pretreatment for samples in comparison with other methods applied to PyC (as charcoal),
297 such as the Acid-Base-Acid processing protocol. This result is promising and warrants further
298 investigation.

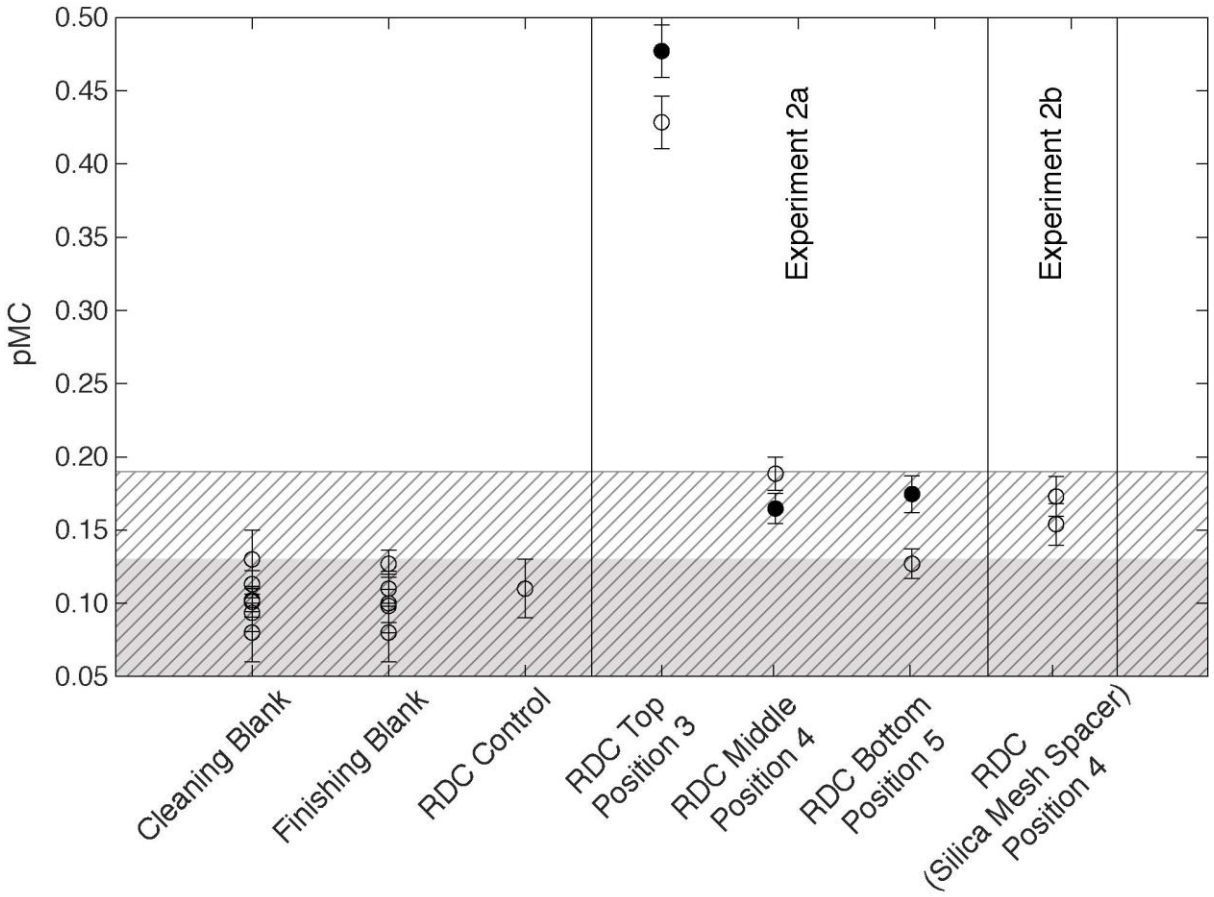
299 3.3 Experiment 2b: Assessment of downstream contamination potential by radiocarbon

300 To assess the potential for transfer of exogenous ^{14}C between samples downstream in the
301 reactor, labile TBM (116.35 pMC) was placed above three samples of RDC. This represents a
302 'worst case' scenario, where radiocarbon dead material is contaminated with modern carbon,
303 and where the source of the modern contamination is completely labile in the reactor. RDC
304 samples positioned directly below the TBM returned 0.477 and 0.429 ±0.02 pMC
305 respectively (equivalent to 42938 ±150 and 43801 ±167 ^{14}C years, Fig 4) in duplicate runs.
306 The radiocarbon content of the RDC samples reduces further down the profile in the reactor

307 to ≤ 0.189 ±0.01 (≤ 50398 ±252 ^{14}C years) which is within error of the quartz tube
308 background (at position 4 in Fig 2b) and below background levels after that.

309 The addition of a silica mesh spacer between the TBM and RDC samples (see fig 2c for
310 placement), reduces the transfer of modern carbon downstream to levels that are not
311 distinguishable from the process background. These results should be interpreted as 'worst
312 case' given the very high loading of 100% labile (under HyPy conditions) and 'modern' (in
313 ^{14}C terms) carbon that was used as the 'contaminant' in this case. Positioning a radiocarbon-
314 dead sample below a significant amount of modern contaminant (~20-45mg of modern
315 carbon), at worst, returns a value of <0.5 pMC however the use of a silica mesh spacer
316 between samples is sufficient to ensure that no subsequent samples are affected. Therefore,
317 even when pretreating 'worst case' samples for radiocarbon analysis via HyPy (i.e. high
318 levels of modern labile C that has a significantly different ^{14}C age to other material analysed
319 in the same run), these results indicate that it is acceptable to process up to 3 samples
320 simultaneously in a 45 minute HyPy run when interspersed with silica mesh spacers.

321



322

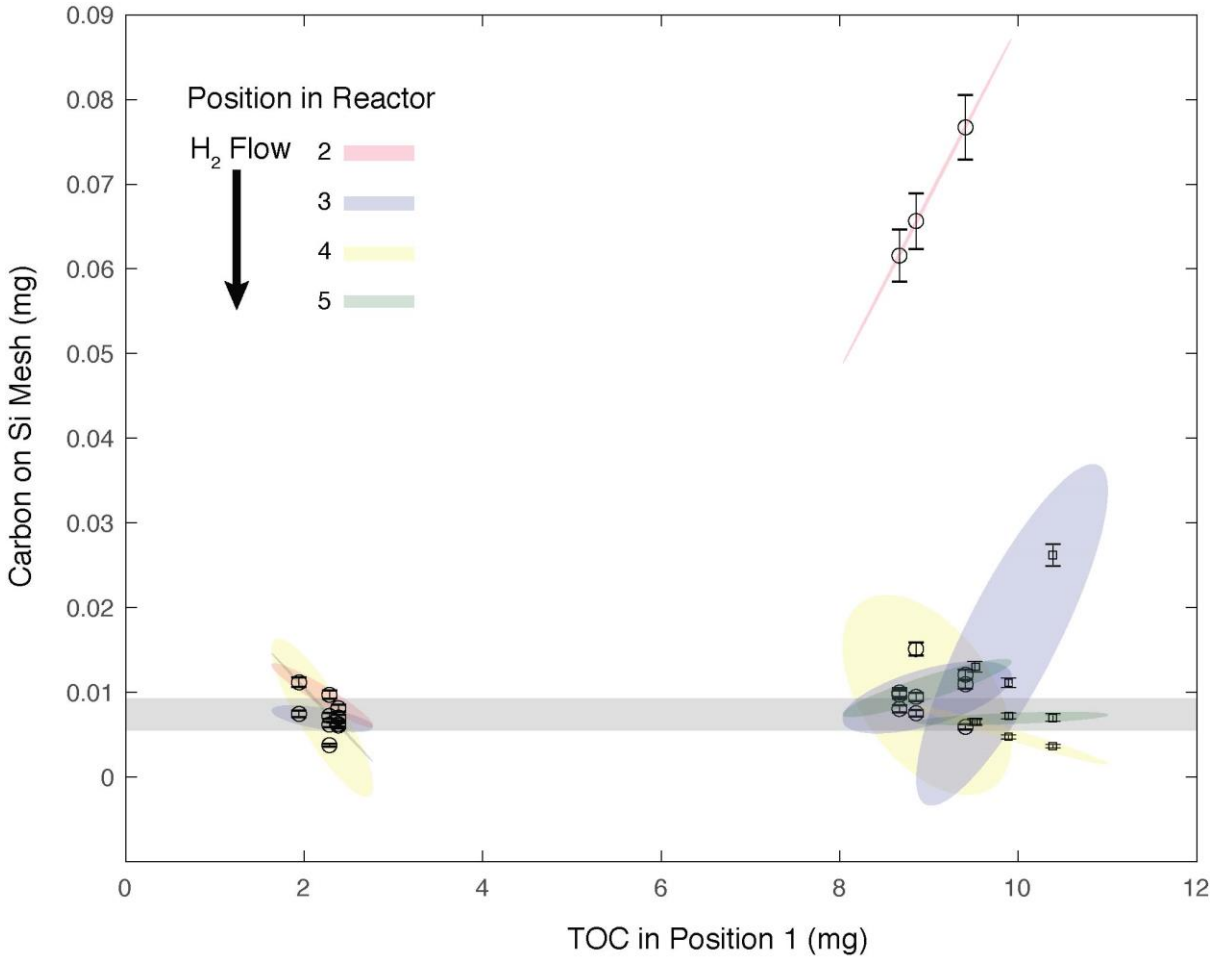
323 **Figure 4.** *Radiocarbon results in pMC (without background correction) for experiment 2*
324 *indicating 2σ error. Dashed shading indicates the accepted NERC Radiocarbon laboratory*
325 *internal quartz tube process background value of 0.17 ±0.02 pMC, grey shading indicates the*
326 *'HyPy' process background value of 0.10 ±0.06 pMC for comparison. RDC top, middle and*
327 *bottom indicates the location of the radiocarbon dead charcoal below the barley mash vessel*
328 *(116.35 pMC), no silica mesh spacer was used for these samples, filled (unfilled) circles*
329 *distinguish the experiments using ~100mg (~50mg) of barley mash.*

330 3.4 Experiment 3: Assessment of downstream contamination potential by labile C

331 To quantify the amount of labile carbon transferred downstream to vessels lower in the
332 reactor column, a vessel containing labile carbon was placed in position 1 atop silica mesh
333 spacers positioned downstream (refer to fig 2d for placement). The silica mesh was analysed
334 for C abundance immediately following the HyPy run (see Fig 5). The silica mesh

335 background (blank) was determined to be 0.005 ±0.004 mg carbon. The high TOC
336 experiment resulted in a transfer of 0.07 ±0.02 mg carbon (0.67% of total labile carbon) from
337 the high organic carbon sample in position 1 onto the silica mesh directly below (at position
338 2). The addition of an empty vessel (empty spacer) at position 2 between the high TOC
339 sample and the silica mesh below (at position 3), reduced the transfer to 0.02 ±0.02 mg
340 (0.09% of total labile carbon), which is an 86.6% reduction in the amount of C transfer from
341 the sample above and within ~10% of the sample in the same position (position 3) in the
342 previous high TOC experiment.

343 All silica mesh samples in the low TOC experiment are below the measured background
344 (effectively 0% carbon). Silica mesh in positions 3, 4 and 5 in the high TOC experiment and
345 positions 4 and 5 in the spacer experiment are also below background (effectively 0%
346 carbon). In practice, it is unlikely that a 'typical' sample in a large vessel could accommodate
347 >10mg of carbon. In this worst case scenario, there is a limited effect on the sample
348 immediately beneath an organic-rich sample and little to no effect on the remaining samples
349 downstream. This effect is small (i.e. <0.73% carbon is transferred from the sample above to
350 the sample below) and can easily be addressed with the interposition of an empty spacer (or
351 silica mesh spacer).



352

353 **Figure 5.** *Milligrams of carbon transferred onto the silica mesh samples positioned below a*
354 *low TOC organic (BCM) or a high TOC organic (Sugarcane Leaves) (open circles) and a*
355 *high TOC organic material with an empty spacer at position 2 acting as a spacer (open*
356 *squares). Ellipses indicate the 95% confidence interval at each position (coloured shading).*
357 *Grey shading indicates the background silica mesh blank value of 0.005 ±0.004 mg (2σ).*

358 3.5 Experiment 4: Assessment of downstream contamination potential by $\delta^{13}C$

359 Isotopically dissimilar labile carbon-rich material was placed in position 1 to test whether
360 the $\delta^{13}C$ value of a sample below (in position 2) is affected. The C4(C3) soils below the
361 C3(C4) organics in position 1, returned $\delta^{13}C$ values which were significantly different from
362 the control (p value = 0.01 and 0.03 respectively), lowering (increasing) the δ^{13}PyC of the
363 material below relative to the C4(C3) control (see fig 6). This effect is minimal in samples

with higher PyC% (fig 6b), and slightly more noticeable in samples with less PyC% (fig 6a) resulting in an offset of 0.3‰ and 0.6‰; and 0.06% and 0.01%. The addition of an amorphous silica mesh spacer between the two materials negates any measurable transfer of labile carbon from the organic material above onto the soil sample below (t = -1.33 and 0.86 respectively). In short, HyPy treatment of multiple isotopically divergent materials (>10‰ in this case) can offset δ^{13}PyC in downstream samples by as much as 0.6‰ however, this can be negated by the use of a silica mesh spacer.

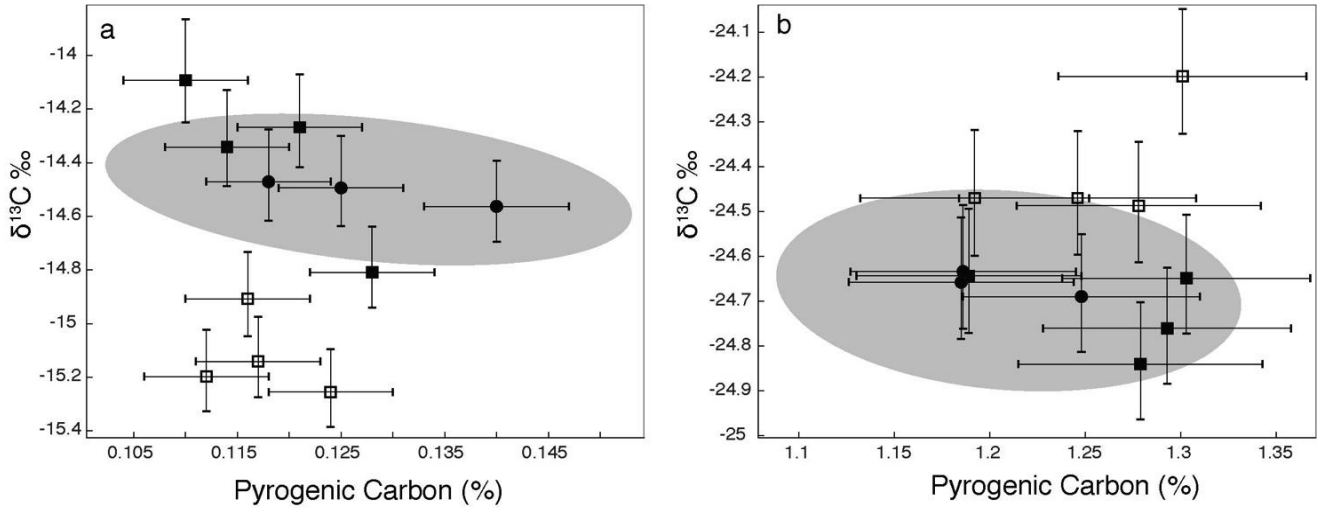


Figure 6. *δ^{13}PyC and PyC(%) of C4(C3) soil samples in sample vessels positioned below C3(C4) organics a(b) in position 1. Black circles denote the C4(C3) controls, black squares indicate that a silica mesh spacer was placed between the organic and soil sample vessels, open squares indicate that a silica mesh spacer was not used. Grey shading indicates the 95% confidence interval of the control samples C4 soil (A) and C3 soil (B) respectively, δ^{13}PyC and PyC (%) error determined as per Wurster et al [31].*

4. Conclusions

HyPy has previously been shown to produce accurate and precise determinations of radiocarbon abundance [12,13] and δ^{13}C value of a well defined component of PyC [31,6]. This study has found that there is a ~7 cm zone in the HyPy reactor where reactor conditions

are identical and thus multiples of 7 (small) or 5 (large) samples, in bespoke borosilicate vessels, can be run in tandem within a single 45 minute HyPy run, with a precision (2σ) better than 0.08% PyC and 0.6‰ δ^{13}PyC. Experiments using labile carbon-rich samples immediately above samples of highly divergent isotope composition were able to detect trace cross-contamination. However, radiocarbon dead PyC positioned immediately below 20-45mg of labile carbon with a high radiocarbon content resulted in <0.5 pMC transfer into the radiocarbon dead sample immediately below. Similarly, small transfers were found using samples widely divergent in δ^{13}C value. Cross-contamination only occurred in the sample immediately below the labile carbon source and did not carry downstream to samples lower in the reactor. In all cases, the use of a silica mesh spacer eliminated cross-contamination between samples. Therefore, even when pretreating 'dirty' samples for radiocarbon or stable isotope analysis using HyPy, the results indicate that it is acceptable to process up to 3 samples simultaneously. In addition, lower radiocarbon backgrounds were obtained using the HyPy process in comparison to standard pretreatment protocols at NCRF, suggesting further potential for application as a low blank pretreatment for radiocarbon dating should be further investigated.

The step that currently limits the application of hydrogen pyrolysis for PyC isolation and analysis is the 43 minute temperature ramp to remove labile carbon (and subsequent 23 minute period required for cooling), with only ~7 samples able to be processed in a single day. This study suggests that 3 (with spacers) to 7 (without spacers) samples can be processed in a single run without diminishing accuracy or precision, enabling ~20-50 samples to be processed in a day. This is comparable to the number of samples that can be run by EA-IRMS for stable isotopes in a day, removing the current bottleneck in routine application to larger scale projects where quantification of PyC and determination of isotope composition is required.

Acknowledgements

This research was funded by the Australian Research Council Centre of Excellence for Australian Biodiversity and Heritage (CE170100015) and an Australian Research Council Laureate Fellowship to M.I.B (FL140100044).

References

1 Bird MI, Wynn JG, Saiz G, Wurster CM, McBeath A. The Pyrogenic Carbon Cycle. *Annual Review of Earth and Planetary Sciences.* 2015;43(1):273-298. doi:10.1146/annurev-earth-060614-105038.

2 Santin C, Doerr SH, Kane ES, Masiello CA, Ohlson M, de la Rosa JM, Preston CM, Dittmar T. Towards a global assessment of pyrogenic carbon from vegetation fires. *Glob Chang Biol.* 2016;22(1):76-91. doi:10.1111/gcb.12985.

3 Reisser M, Purves RS, Schmidt MWI, Abiven S. Pyrogenic Carbon in Soils: A Literature-Based Inventory and a Global Estimation of Its Content in Soil Organic Carbon and Stocks. *Frontiers in Earth Science.* 2016;4doi:10.3389/feart.2016.00080.

4 Koele N, Bird M, Haig J, Marimon BH, Marimon BS, Phillips OL, de Oliveira EA, Quesada CA, Feldpausch TR. Amazon Basin forest pyrogenic carbon stocks: First estimate of deep storage. *Geoderma.* 2017;306:237-243. doi:10.1016/j.geoderma.2017.07.029.

5 Sohi SP, Krull E, Lopez-Capel E, Bol R. Chapter 2 - A Review of Biochar and Its Use and Function in Soil. In: Sparks, D. L. ed. *Advances in Agronomy.* Elsevier; 2010:47-82.

6 Wurster CM, McBeath AV, Bird MI. The carbon isotope composition of semi-labile and stable pyrogenic carbon in a thermosequence of C3 and C4 derived char. *Organic Geochemistry.* 2015;81:20-26. doi:10.1016/j.orggeochem.2015.01.008.

7 Hammes K, Schmidt MWI, Smernik RJ, Currie LA, Ball WP, Nguyen TH, Louchouarn P, Houel S, Gustafsson O, Elmquist M, Cornelissen G, Skjemstad JO, Masiello CA, Song J, Peng P, Mitra S, Dunn JC, Hatcher PG, Hockaday WC, Smith DM, Hartkopf-Froeder C, Boehmer A, Luer B, Huebert BJ, Amelung W, Brodowski S, Huang L, Zhang W, Gschwend PM, Flores-Cervantes DX, Largeau C, Rouzaud JN, Rumpel C, Guggenberger G, Kaiser K, Rodionov A, Gonzalez-Vila FJ, Gonzalez-Perez JA, de la Rosa JM, Manning DAC, Lopez-Capel E, Ding L. Comparison of quantification methods to measure fire-derived (black/elemental) carbon in soils and sediments using reference materials from soil, water, sediment and the atmosphere. *Global Biogeochemical Cycles.* 2007;21(3)doi:10.1029/2006gb002914.

8 Knicker H, Almendros G, González-Vila FJ, Martin F, Lüdemann HD. 13C- and 15N-NMR spectroscopic examination of the transformation of organic nitrogen in

plant biomass during thermal treatment. *Soil Biology and Biochemistry.* 1996;28(8):1053-1060. doi:10.1016/0038-0717(96)00078-8.

9 Chang Z, Tian L, Li F, Zhou Y, Wu M, Steinberg CEW, Dong X, Pan B, Xing B. Benzene polycarboxylic acid - A useful marker for condensed organic matter, but not for only pyrogenic black carbon. *Sci Total Environ.* 2018;626:660-667. doi:10.1016/j.scitotenv.2018.01.145.

10 Love GD, Snape CE, Carr AD, Houghton RC. Release of covalently-bound alkane biomarkers in high yields from kerogen via catalytic hydropyrolysis. *Organic Geochemistry.* 1995;23(10):981-986. doi:10.1016/0146-6380(95)00075-5.

11 Craig OE, Love GD, Isaksson S, Taylor G, Snape CE. Stable carbon isotopic characterisation of free and bound lipid constituents of archaeological ceramic vessels released by solvent extraction, alkaline hydrolysis and catalytic hydropyrolysis. *Journal of Analytical and Applied Pyrolysis.* 2004;71(2):613-634. doi:10.1016/j.jaap.2003.09.001.

12 Ascough PL, Bird MI, Brock F, Higham TFG, Meredith W, Snape CE, Vane CH. Hydropyrolysis as a new tool for radiocarbon pre-treatment and the quantification of black carbon. *Quaternary Geochronology.* 2009;4(2):140-147. doi:10.1016/j.quageo.2008.11.001.

13 Ascough PL, Bird MI, Meredith W, Wood RE, Snape CE, Brock F, Higham TFG, Large DJ, Apperley DC. Hydropyrolysis: Implications for Radiocarbon Pretreatment and Characterization of Black Carbon. *Radiocarbon.* 2010;52(3):1336-1350. doi:10.1017/S0033822200046427.

14 Meredith W, Ascough PL, Bird MI, Large DJ, Snape CE, Sun Y, Tilston EL. Assessment of hydropyrolysis as a method for the quantification of black carbon using standard reference materials. *Geochimica Et Cosmochimica Acta.* 2012;97:131-147. doi:10.1016/j.gca.2012.08.037.

15 Robert AM, Grotheer H, Greenwood PF, McCuaig TC, Bourdet J, Grice K. The hydropyrolysis (HyPy) release of hydrocarbon products from a high maturity kerogen associated with an orogenic Au deposit and their relationship to the mineral matrix. *Chemical Geology.* 2016;425:127-144. doi:10.1016/j.chemgeo.2016.01.028.

16 Rombola AG, Fabbri D, Meredith W, Snape CE, Dieguez-Alonso A. Molecular characterization of the thermally labile fraction of biochar by hydropyrolysis and pyrolysis-GC/MS. *Journal of Analytical and Applied Pyrolysis.* 2016;121:230-239. doi:10.1016/j.jaap.2016.08.003.

17 Sephton MA, Love GD, Meredith W, Snape CE, Sun CG, Watson JS. Hydropyrolysis: A new technique for the analysis of macromolecular material in meteorites. *Planetary and Space Science.* 2005;53(12):1280-1286. doi:10.1016/j.pss.2005.06.008.

18 Foereid B, Lehmann J, Wurster C, Bird M. Presence of Black Carbon in Soil due to Forest Fire in the New Jersey Pine Barrens. *Journal of Earth Science and Engineering.* 2015;5(2):91-97. doi:10.17265/2159-581x/2015.02.001.

19 Santin C, Doerr SH, Merino A, Bucheli TD, Bryant R, Ascough P, Gao X, Masiello CA. Carbon sequestration potential and physicochemical properties differ between wildfire charcoals and slow-pyrolysis biochars. *Sci Rep.* 2017;7(1):11233. doi:10.1038/s41598-017-10455-2.

20 Wurster CM, Saiz G, Schneider MPW, Schmidt MWI, Bird MI. Quantifying pyrogenic carbon from thermosequences of wood and grass using hydrogen pyrolysis. *Organic Geochemistry.* 2013;62:28-32. doi:10.1016/j.orggeochem.2013.06.009.

21 Meredith W, McBeath A, Ascough P, Bird MI. Analysis of biochars by hydropyrolysis (HyPy). In: Singh, B., Camps Arbestain, M. & Lehmann, J. eds. *Biochar: A Guide to Analytical Methods.* Clayton: CRC Press; 2017.

22 Grotheer H, Greenwood PF, McCulloch MT, Böttcher ME, Grice K. δ34S character of organosulfur compounds in kerogen and bitumen fractions of sedimentary rocks. *Organic Geochemistry.* 2017;110:60-64. doi:10.1016/j.orggeochem.2017.04.005.

23 Saiz G, Goodrick I, Wurster C, Nelson PN, Wynn J, Bird M. Preferential Production and Transport of Grass-Derived Pyrogenic Carbon in NE-Australian Savanna Ecosystems. *Frontiers in Earth Science.* 2018;5(1):115. doi:10.3389/feart.2017.00115.

24 Field E, Marx S, Haig J, May JH, Jacobsen G, Zawadzki A, Child D, Heijnis H, Hotchkis M, McGowan H, Moss P. Untangling geochronological complexity in organic spring deposits using multiple dating methods. *Quaternary Geochronology.* 2018;43:50-71. doi:10.1016/j.quageo.2017.10.002.

25 Zhang XY, Huang DS, Deng H, Snape C, Meredith W, Zhao Y, Du Y, Chen X, Sun YG. Radiocarbon dating of charcoal from the Bianjiashan site in Hangzhou: New evidence for the lower age limit of the Liangzhu Culture. *Quaternary Geochronology.* 2015;30:9-17. doi:10.1016/j.quageo.2015.07.001.

26 Llorente M, Turrion MB, Glaser B. Rapid and economical quantification of black carbon in soils using a modified benzene polycarboxylic acids (BPCA) method. *Organic Geochemistry.* 2018;115:197-204. doi:10.1016/j.orggeochem.2017.11.005.

27 Gulliksen S, Scott M. Report of the TIRI workshop, Saturday 13 August 1994. *Radiocarbon.* 1995;37(2):820-821. doi:10.1017/S0033822200031404.

28 Rozanski K, Stichler W, Gonfiantini R, Scott EM, Beukens RP, Kromer B, Van Der Plicht J. The IAEA 14C Intercomparison Exercise 1990. *Radiocarbon.* 2016;34(3):506-519. doi:10.1017/s0033822200063761.

29 Bird MI, Levchenko V, Ascough PL, Meredith W, Wurster CM, Williams A, Tilston EL, Snape CE, Apperley DC. The efficiency of charcoal decontamination for

radiocarbon dating by three pre-treatments - ABOX, ABA and hypy. *Quaternary Geochronology.* 2014;22:25-32. doi:10.1016/j.quageo.2014.02.003.

30 Love GD, McAulay A, Snape CE, Bishop AN. Effect of process variables in catalytic hydropyrolysis on the release of covalently bound aliphatic hydrocarbons from sedimentary organic matter. *Energy & Fuels.* 1997;11(3):522-531. doi:10.1021/ef960194x.

31 Wurster CM, Lloyd J, Goodrick I, Saiz G, Bird MI. Quantifying the abundance and stable isotope composition of pyrogenic carbon using hydrogen pyrolysis. *Rapid Commun Mass Spectrom.* 2012;26(23):2690-2696. doi:10.1002/rcm.6397.

32 Slota PJ, Jull AJT, Linick TW, Toolin LJ. Preparation of Small Samples for 14C Accelerator Targets by Catalytic Reduction of CO. *Radiocarbon.* 1987;29(2):303-306. doi:10.1017/s0033822200056988.

33 Stuiver M, Polach HA. Discussion Reporting of 14C Data. *Radiocarbon.* 2016;19(3):355-363. doi:10.1017/s0033822200003672.

Table 1. *Consensus values for in-house materials used in experiments to assess variation in measured PyC abundance and $\delta^{13}C$ value when combining multiple samples during a single hydropyrolysis run (error is reported as 2σ).*

Soil Samples		TOC (%)	$\delta^{13}C$	PyC (%)	$\delta^{13}PyC$
	BC Mollisol (BCM)	2.04 ±0.02	-25.57 ±0.2	0.25 ±0.1	-26.47 ±0.6
C3	SAN2 Surface	13.44 ±0.6	-25.73 ±0.5	1.18 ±0.1	-24.66 ±0.2
C4	AGR	1.51 ±0.1	-16.51 ±0.1	0.15 ±0.01	-14.74 ±0.3
Labile Organic Samples					
C3	Rainforest Leaves	44.08 ±4.4	-33.47 ±0.1	N/A	N/A
C4	Sugarcane Leaves	42.11 ±4.2	-11.86 ±0.3	N/A	N/A

Table 2. *Position experiment summary statistics*

		PyC (%)		δ^{13}PyC (‰)	
BCM	Position	μ	2σ	μ	2σ
	1	0.3	0.04	-26.2	0.3
	2	0.3	0.12	-26.2	0.4
	3	0.2	0.02	-26.6	0.4
	4	0.2	0.04	-26.7	0.3
	5	0.2	0.04	-26.7	0.2
	6	0.2	0.08	-26.4	0.9
	7	0.3	0.08	-26.7	0.2
	8	0.5	0.18	-26.5	0.3
	9	0.9	0.36	-26.2	1
AGR	1	0.2	0.01	-14.8	0.4
	2	0.2	0.01	-14.7	0.2
	3	0.1	0.03	-14.6	0.5
	4	0.2	0.04	-14.7	0.4
	5	0.1	0.02	-14.9	0.1
	6	0.2	0.07	-15.3	0.8